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Systems toxicology meta-analysis—From aerosol exposure to nanotoxicology

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Abstract

Systems toxicology marks an important stage in the evolution of toxicology. It combines the insights from traditional toxicology end points, high-throughput data, and quantitative analysis of large cause-and-effect molecular network models that provide the most mechanistic information in the interpretation of high-throughput data. Here, we show an example on how pulmonary causal biological network models can be used in a meta-analysis of independent studies on engineered nanomaterials to gain mechanistic insight into the similarities and differences of the ways the engineered nanomaterials impact biological processes in the mouse lung. Meta-analyses using the lung network models could be used in various toxicological applications to find underlying trends in response to exposures, derive compound-specific mechanistic signatures, and translate between species.

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Keywords

Systems toxicology, Meta-analysis, Causal biological network model, Inhalation exposure, Engineered nanomaterials.

Abbreviations

BAL, bronchoalveolar lavage; ENM, engineered nanomaterials; CNT, carbon nanotube; TiO_2NP, nano titanium dioxide; BEL, biological expression language; NPA, network perturbation amplitude; THS, Tobacco Heating System; SD, sanding dust.

Introduction

In their recent review, Smirnova et al. [1] state that "there are two systems biology/toxicology approaches—one that is computational and one that is experimental—and they complement each other in addressing the complexity of the organism". The computational systems toxicology approach is largely about using or developing algorithms to best exploit systematically and comprehensively generated biological data sets; a recent review by Dann et al. outlines the various approaches that can be used to extract predictive information about compound-induced toxicity from gene expression data [2]. The review also details resources, such as the Comparative Toxicogenomics Database [3], DrugMatrix database [4], and TG-GATE [5], that aim to associate gene expression with toxicity [2]. In addition to the aforementioned toxicogenomics databases, the Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/) and ArrayExpress (https://www.ebi.ac.uk/arrayexpress/) offer a number of data sets on toxicological exposures in various experimental systems and conditions to be explored by the computational scientists. Consequently, the scientific literature provides many examples of mechanistic insights obtained from omics data, ranging from drug-induced liver injury to toxicity of modern munitions used by the US military [6–8].

A meta-analysis uses data from independent but related studies to generalize conclusions, to increase statistical sensitivity, or to understand why conclusions from different studies diverge [9]. Meta-analysis has been used successfully for the identification of dysregulated genes that are associated with pathology induced by nephrotoxic drugs [10], carcinogenic effects of a compound [11], or individual risk for adverse drug reactions [12].

Meta-analysis can also be used for the grouping of compounds based on their biological impact. For example, stress response is a common defense mechanism in response to chemical exposures. Schutter et al. [13] set out to find commonalities in transcript regulation in response to chemical stress in zebrafish by combining microarray data from 33 studies with exposure to 60 different chemicals. In addition to looking into the low number of genes that were significantly differentially expressed in each individual treatment, the authors took advantage of the effect size analysis, followed by functional enrichment analysis, to derive conclusions about a uniform stress response. As a result,

22 chemicals of the 40 included in the effect size analysis were grouped into three broad groups with similar modes of action [13].

A burning question in toxicology research is whether the toxicological predictions from rodent models can be translated to humans. In particular, frequent drug attrition due to undesirable toxicity is often because of the fact that the toxicity biomarkers identified are limited to certain species and experimental systems. Kim et al. used more than 6000 samples in a meta-analysis to characterize the toxicity of drugs in liver, kidney, and multiple organ specimens. As expected, the drugs tested exhibited time- and dose-dependent tissue-specific toxicity. Feature reduction followed by gene ontology and protein−protein interaction network analysis yielded a prediction model that performed well when tested computationally and experimentally in human cells [14].

By systemically comparing available gene expression data sets from both rodent and human bronchoalveolar lavage (BAL) for acute lung injury and acute respiratory distress syndrome, Sweeney et al. [15] set forth to find similar expression changes in rodents and humans in response to lung injury. The gene signatures resulting from the integrated multicohort transcriptomic analysis were mapped against kyoto encyclopedia of genes and genomes (KEGG) pathways as well as the animal lung tissue and human BAL fluid gene expression signatures allowing the identification of affected biological pathways and predominant cell types in the samples, respectively. The data were also compared with the Library of Integrated Network-based Cellular Signatures to identify potential therapeutic targets [15].

Owing to the rapidly increasing human exposure to engineered nanomaterials (ENMs), the identification of the adverse effects from the exposure to nanophase materials on living organisms and the environment [16−19] has become a hot topic in toxicology research. Nikota et al. [20] conducted meta-analyses on a number of publicly available studies that aim to assess the health effects of a variety of ENMs of varying properties to understand underlying mechanisms of toxicity via pulmonary exposure in rodents. The major routes of uptake for ENMs are the respiratory tract, the gastrointestinal tract, and the skin; for occupational exposure, the respiratory tract is the most prevalent exposure route. The expression profiles in response to ENM exposure were compared with the lung gene expression profiles of 12 different lung disease models. This approach allowed the identification of disease pathways stimulated in the rodent lung upon nanoparticle exposure as well as differences between carbon nanotube (CNT), carbon black, and nano−titanium dioxide (TiO_2NP) exposure [20]. A very recent example of a meta-analysis in this field integrated independent gene expression data sets for improved statistical power to detect robust and precise differential gene expression in response to exposure to silver nanoparticles [21]. Meta-analysis can also consist of different omics data that are analyzed in an integrated manner. Gioria et al. [22] used proteomics and metabolomics profiling to complement immunochemistry, microscopic analysis, and multiplexed assays to derive true systems toxicology insights into the effects of silver nanoparticles of different sizes.

The aforementioned examples demonstrate how meta-analyses of high-throughput data pave the way to more accurate toxicity predictions. In the next section, we will introduce the value of computational network models in systems toxicology meta-analysis.

Meta-analysis using network biology

Causal biological network models

According to the definition by Sturla et al. [23], systems toxicology is "the integration of classical toxicology with quantitative analysis of large networks of molecular and functional changes occurring across multiple levels of biological organization". In systems toxicology, quantitative systems-wide molecular changes in the context of an exposure are measured, and a causal chain of molecular events linking exposures with adverse outcomes (i.e., functional and apical end points) are deciphered [23]. In line with this definition and apparent from several articles on various use cases, we advocate the use of causal biological network models in high-throughput data interpretation. Over the years, this has become the basis of our systems toxicological assessment, which relies on quantitative comparison of impacted biology between exposures [24]. The causal biological network models are scripted in the Biological Expression Language (https://bel.bio/), which represents scientific findings by capturing relationships between biological entities (such as enzymes and their substrates) into cause-and-effect assertions. A browsable view of recent network models is available in the Causal Biological Networks database (http://causalbionet.com) [25].

A network contains various biological entities (backbone nodes) that are further designed to facilitate the interpretation of numerous gene expression changes that are considered to be triggered by the upstream biological entities embedded in the backbone [26]. The network perturbation amplitude (NPA) algorithm provides a quantification of the backbone nodes, referred to as the "differential network backbone value." For instance, the transcriptional activity of the aryl hydrocarbon receptor is predicted by fold changes in several downstream genes. Typically, backbone values in a network are based on expression changes from hundreds to thousands of genes. These backbone values, together with the

network topology, define the perturbation of the model as a whole [27]. The NPA algorithm and a suite of eight such models are publicly available as R packages and can be downloaded from the GitHub project pages https://github.com/pmpsa-hpc/NPA and https://github.com/pmpsa-hpc/NPAModels.

In particular, the xenobiotic metabolism network model, which was initially published as part of the biological network model suite describing various stress responses in lung cells [28] and further refined to better reflect the three phases of xenobiotic metabolism in lung tissue, has proven suitable for the meta-analysis of the xenobiotic response in the smoker bronchial epithelium [29]. We have shown that a good correlation of individual studies on bronchial brushings from smokers can be obtained using the differential backbone values of the xenobiotic metabolism network model, while the correlation of the gene expression remained moderate. Moreover, the correlation remained when organotypic bronchial cultures were exposed to cigarette smoke and compared with human brushing data [29,30].

The network-based systems toxicology approach has been extensively used in the assessment of the Tobacco Heating System (THS) 2.2, which is one of the recent advancements in the development of novel reduced-risk tobacco products [31]. In combination with important toxicological end points in various experimental systems, the approach quantitatively demonstrated the reduced impact of THS 2.2 exposure compared with cigarette smoke [32–41].

The causal biological network models were recently also used in a meta-analysis of *in vitro* assessment studies that focused on three human organotypic cultures of the aerodigestive tract (buccal, bronchial, and nasal epithelia) exposed to cigarette smoke and the aerosol from THS 2.2. Thousands of simultaneously measured data points were put into the context of known biological processes, and their activation/perturbation/disruption were deciphered using computational evaluation strategies and causal network modeling. Using the tools of systems toxicology and integrated analysis across various levels of biological organization, the molecular stress responses, together with functional and structural changes, were investigated directly in nonclinical systems, where exposed cells/tissues are readily available [42].

Use case: meta-analysis of nanotoxicology studies using the network approach

The purpose of this use case is to demonstrate how the causal biological network models can be used to gain insight into the molecular mechanisms that are impacted in the lungs of mice exposed to ENMs. We have used transcriptomic data from some of the studies (GSE29042, GSE35193, GSE55286, and GSE60797-GSE60800) reported in the meta-analysis conducted by Nikota et al. [20] and have focused on the mechanisms impacted after one day or 28 days postexposure. The studies used are summarized in Table 1 and include the testing of CNTs [43,44], carbon black [45], and TiO_2NPs in a free form or embedded in sanding dust (SD) [46].

The causal biological network models used here were built for lung biology [47–51] and have mostly been used to assess respiratory toxicants, such as cigarette smoke [32–42]. Instead of limiting the biology to lung disease models, they cover a wide range of biological processes and thus facilitate unbiased data interpretation in context of *a priori* biological knowledge. Moreover, when the number of differentially expressed genes that overlap between the data sets is low, the network approach can overcome limitations of traditional pathway analyses.

Figure 1 shows the workflow of converting publicly available gene expression series matrix to NPA of selected network models. For the one-day post-exposure time point, we chose biological network models that represent acute stress response and mechanisms typical in cell fate decisions and proliferative signaling. Figure 2A shows the NPAs of the selected network models in response to ENM exposure. Generally, the CNTs triggered stronger network perturbation compared with the carbon black and TiO_2NPs at the one-day post-exposure time point. The impact of SD on several networks was weaker than that of SD-TiO_2NP20.6, indicating the additional contribution of the TiO_2NP to this impact. However, the impact of TiO_2NP10.5 + 38 was weaker than that of the SD alone.

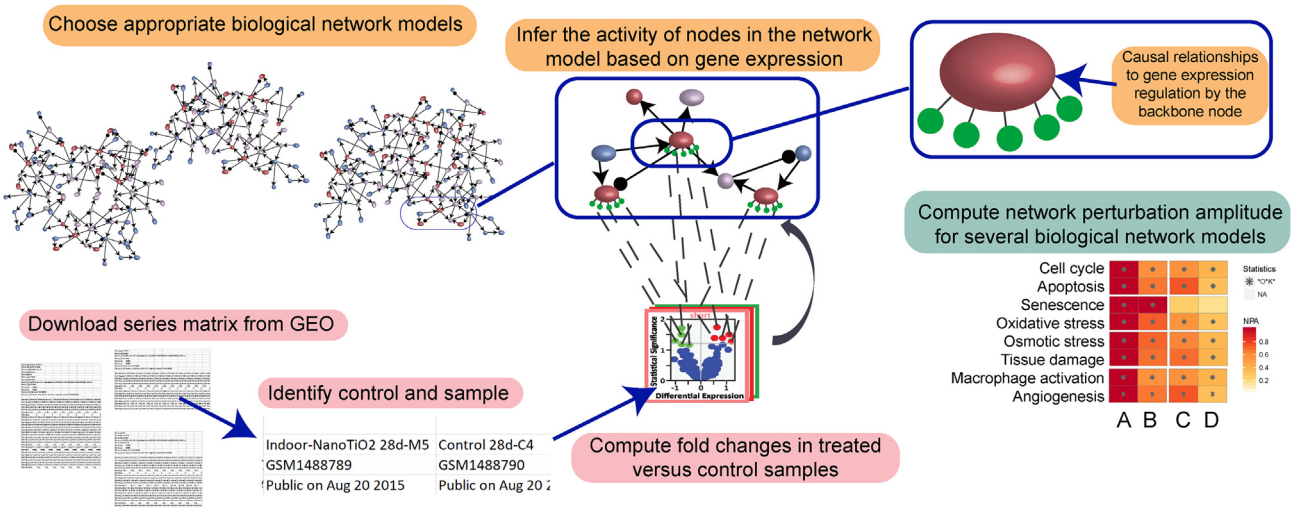
Further insights into how the different nanomaterials perturbed the lung network models can be obtained from the leading node analysis. Leading nodes are the entities in the model backbone that are inferred to be most impacted in response to the exposure and contribute collectively to 80% of the NPA [27]. Moreover, while the NPA heatmap provides a quantitative view of the network impact across the experiments, the leading node analysis shows the directionality of the inferred impact on each node. Figure 2B shows an extract of the cell cycle network model with the leading nodes for SD-TiO_2NP20.6, SD, TiO_2NP38, and TiO_2NP10.5 one day after exposure. Interestingly, the core cell cycle molecules were inferred to be regulated in different directions in response to these TiO_2NPs, indicating a divergent early response to the insult by the SD-embedded and free TiO_2NPs in mouse lung. This could also be observed in the context of the senescence and apoptosis networks that share many mechanisms also present in the cell cycle network model (not shown). While the signal was weaker, the directionalities of

Table 1 Publicly available transcriptomics data sets used in the meta-analysis.

Data set ID	Study title	Type of nanomaterial	Name	Exposure	Reference
GSE29042	Expression profiling of mice exposed to multiwalled carbon nanotubes	Multiwalled carbon nanotubes	CNT Mitsui 7	Single pharyngeal aspiration	[43]
GSE55286	Transcriptomic analysis of mouse lung tissue exposed to two multiwalled carbon nanotubes (NRCWE-26 and NM-401)	Multiwalled carbon nanotubes	NM-401, CNTLarge and NRCWE-26, CNTSmall	Single intratracheal instillation	[44]
GSE35193	Pulmonary gene expression in C57BL/6 mice intratracheally instilled with Printex 90 carbon black nanoparticles	Carbon black nanoparticles	Printex 90	Single intratracheal instillation	[45]
GSE60797	Transcriptional profiling to identify physical–chemical properties detrimental to nanomaterial-induced pulmonary response (part 1)	Sanding dust	SD-TiO_2NP20.6[a] and SD	Single intratracheal instillation	[46]
GSE60798	Transcriptional profiling to identify physical––chemical properties detrimental to nanomaterial-induced pulmonary response (part 2)	Free nano titanium dioxide	TiO_2NP10[a] and TiO_2NP10+[a] (positively charged)	Single intratracheal instillation	[46]
GSE60799	Transcriptional profiling to identify physical––chemical properties detrimental to nanomaterial-induced pulmonary response (part 3)	Free nano titanium dioxide	TiO_2NP38[a] and TiO_2NP10.5[a]	Single intratracheal instillation	[46]
GSE60800	Transcriptional profiling to identify physical––chemical properties detrimental to nanomaterial-induced pulmonary response (part 4)	Sanding dust	SD-TiO_2NP38[a] and SD-TiO_2NP10.5 + 32[a]	Single intratracheal instillation	[46]

SD, sanding dust; CNT, carbon nanotube; TiO_2NP, nano–titanium dioxide.
[a] Value refers to the particle size in nm.

Figure 1



The workflow from publicly available gene expression data to NPA. GEO, Gene Expression Omnibus.

the network node regulation in response to SD aligned with those in response to SD-TiO_2NP20.6.

To score the data from the 28-day post-exposure time point, we chose network models representing inflammation-related and tissue repair–related processes to evaluate longer term effects that could pose a disease risk after single exposure. These network models responded strongest to the exposure to NRCWE-26 and Small CNT, and TiO_2NP10 + was the only TiO_2NP that triggered similar or higher network perturbation scores as the CNTs. The impact on networks was very weak in response to the SD and SD-TiO_2NPs; however, the weakest overall response was observed in response to TiO_2NP38 (Figure 3A). Many inflammation-related processes, including B-cell signaling, dendritic cell signaling, Th17 signaling, tissue damage, mast cell activation, and megakaryocyte differentiation, were not perturbed at 28 days in response to TiO_2NP38 and TiO_2NP10.5 (Figure 3A). With the exception of SD-TiO_2NP10.5 + 38, all analyzed materials, including the SD alone, induced at least a low level of perturbation of the fibrosis network model. Interestingly, Th1-Th2 signaling was impacted not only by the CNTs but also by carbon black, SD, SD-TiO_2NP20.6, SD-TiO_2NP10.5 + 38, TiO_2NP10+, and TiO_2NP38, albeit to a lesser extent. Figure 3B shows a graphical presentation of the top leading nodes and individual scores for the different CNTs on the epithelial innate immune activation network model, highlighting the inferred upregulation of IL1/Myd88/Nfkb signaling.

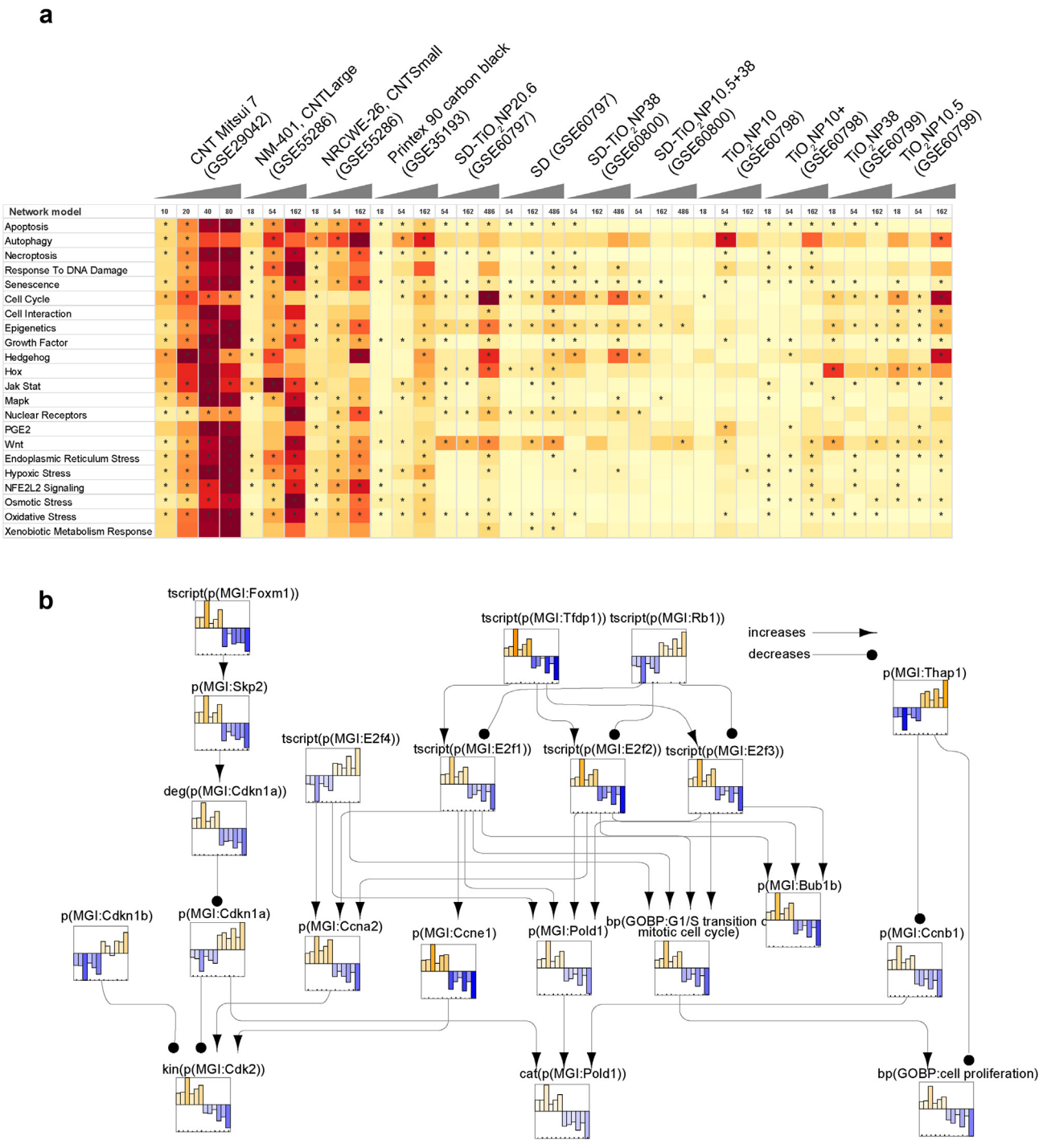
In their analysis, Nikota et al. [20] showed a separate clustering of biological response to TiO_2NPs and CNTs and that only the CNTs clustered with the disease models. With the sensitive network approach, we could dissect a very early divergent response to SD and free TiO_2NPs in the context of the cell cycle network model. Moreover, we have found that many inflammation-related and tissue repair–related processes were also impacted in response to the carbon black and TiO_2NPs, and even SD, without any ENMs. Although many more insights could be derived from the current analysis, these examples should give a sense of what can be achieved using the causal biological network models to interpret toxic effects of nanomaterials in the mouse lung.

Expert opinion

There is an ever-increasing number of omics data sets testing chemical toxicity, and meta-analyses are necessary to obtain a coherent understanding of the biological impact of exposures. While conducting meta-analysis, it is often necessary to reprocess the omics data from individual studies, because researchers may use different omics technology platforms and normalization methods. Additional challenges include heterologous exposure settings (organ and species) as well as different exposure times and concentrations that need to be taken into consideration and controlled during the analysis Figure 4A [9]. There is also a lack of standardization in exposure and biological systems used, and meta-analysis across large-scale systems toxicology data sets will ultimately enable robust identification of the impacted mechanisms and subsequently facilitate risk assessment.

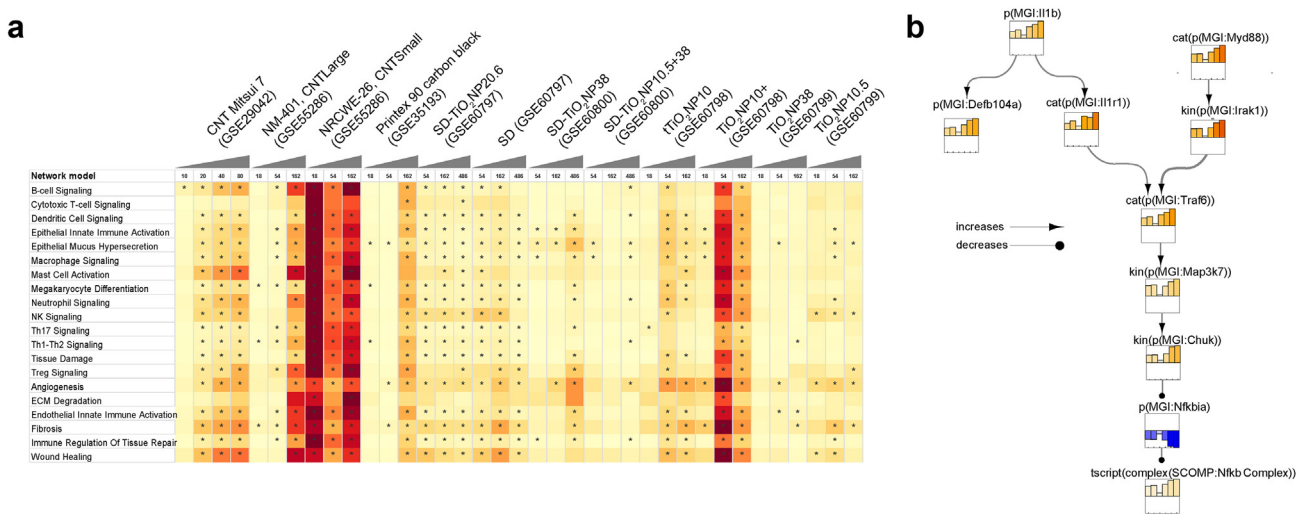
Nanoparticles do not interact with or bind to a single type of macromolecule, but they perturb multiple pathways and influence cellular processes, such as

Figure 2



NPA in response to nanoparticle exposure using pulmonary causal biological network models. **(a)** The series matrix of the gene expression data was downloaded from the GEO, and the gene expression fold changes were computed for each contrast (i.e., treatment versus control). The data at one day postexposure was scored against causal biological network models as described in Ref. [27]. A network is considered as perturbed if, in addition to the significance of the NPA score with respect to the experimental variation, the two companion statistics (O and K), derived to inform on the specificity of the NPA score with respect to the biology described in the network, are significant. *O and K statistic *p*-values below 0.05 and NPA significant with respect to the experimental variation. The number on the top of each column of the heat map indicates the amount used in μg. **(b)** The leading node analysis of after one day exposure to SD-TiO2NP20.6, SD, TiO2NP38, and TiO2NP10.5. Extract of the cell cycle network model containing connected nodes that were common leading nodes in response to the selected nanomaterials. The backbone NPA values with directionalities of inferred regulation are shown as bar graphs for each node: (1–3) SD-TiO2NP20.6 (54, 162, or 486 μg), (4–6) SD (54, 162, or 486 μg), (7–9) TiO2NP38 (18, 54, and 162 μg), and (10–12) TiO2NP10.5 (18, 54, and 162 μg). Orange/red bars indicate inferred upregulation, and blue bars indicate inferred downregulation. Each BEL term

Figure 3



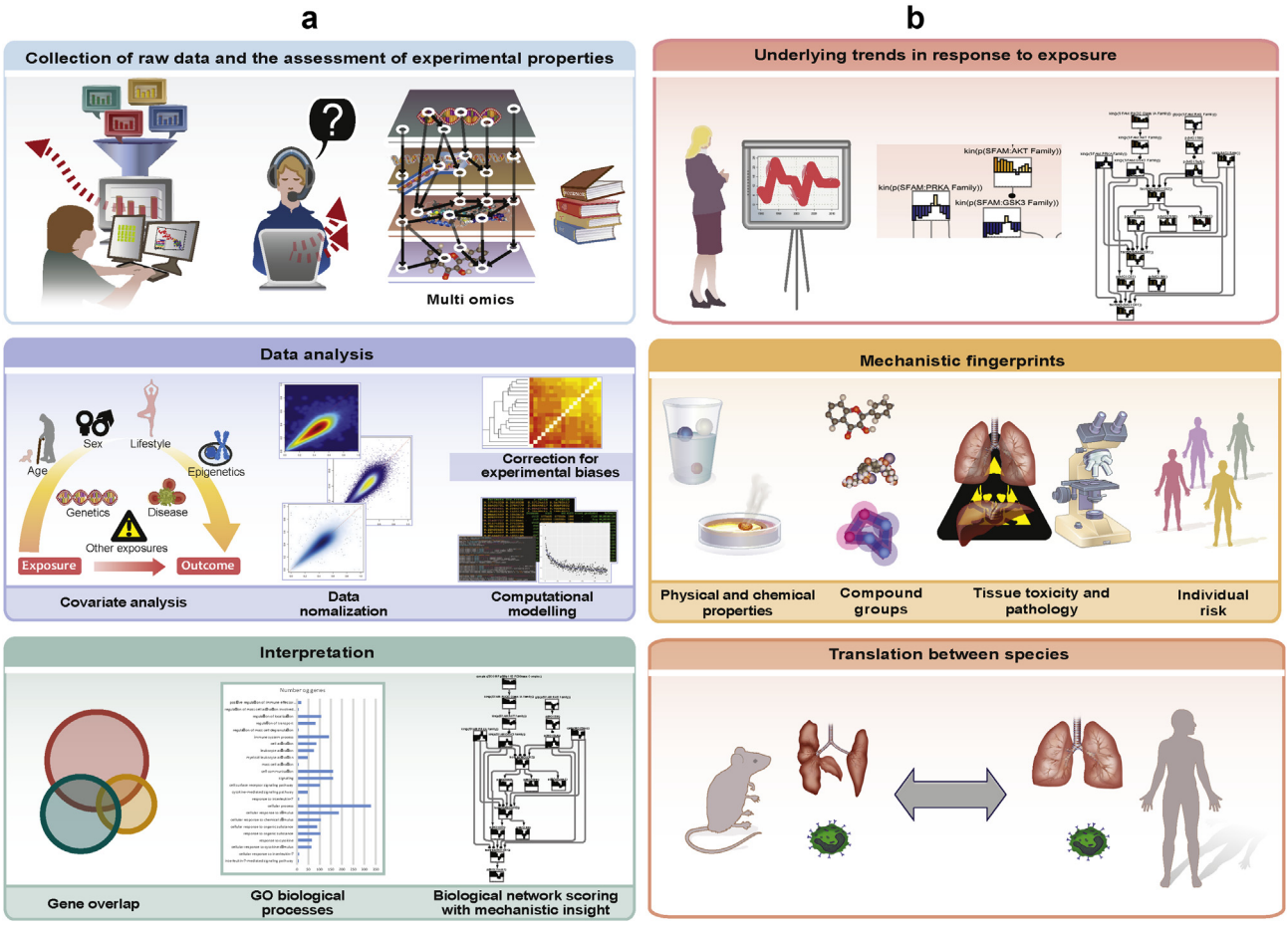
NPA in response to nanoparticle exposure using pulmonary causal biological network models. **(a)** The series matrix of the gene expression data was downloaded from the GEO, and the gene expression fold changes were computed for each contrast (i.e., treatment versus control). The data from 28 days postexposure was scored against causal biological network models as described in Ref. [27]. A network is considered as perturbed if, in addition to the significance of the NPA score with respect to the experimental variation, the two companion statistics (O and K), derived to inform on the specificity of the NPA score with respect to the biology described in the network, are significant. *O and K statistic *p*-values below 0.05 and NPA significant with respect to the experimental variation. The number on the top of each column of the heat map indicates the amount used in μg. **(b)** The leading node analysis 28 days after exposure to CNT Mitsui 7, NM-401, CNTLarge, NRCWE-26, and CNTSmall. Extract of the epithelial innate immune activation network model containing connected nodes that were common leading nodes in response to the selected nanomaterials. The backbone NPA values with directionalities of inferred regulation are shown as bar graphs for each node: (1–2) CNT Mitsui 7 (40 and 80 μg), (3–4) NM-401, CNTLarge (54 and 162 μg), and (5–6) NRCWE-26, CNTSmall (54 and 162 μg). Orange/red bars indicate inferred upregulation, and blue bars indicate inferred down-regulation. Each BEL term contains the molecular function (p, protein abundance; tscript, transcriptional activity; deg, degradation; kin, kinase activity; cat, catalytic activity; bp, biological process) and the namespace definition (MGI, mouse genome informatics; GOBP, gene ontology biological process; SCOMP, Selventa complex). The entire epithelial innate immune activation network model can be downloaded from causalbionet.com, and the BEL terminology can be found in https://bel.bio/. NPA, network perturbation amplitude; BEL, biological expression language; GEO, Gene Expression Omnibus.

proliferation, apoptosis, inflammation, and membrane integrity. In addition to ENMs, the investigation of ultrafine particulate matter (PM0.1) and other nanomaterials is equally important [52,53]. The identification of new sensitive markers for the toxicity associated with particle matter of any size is needed, because exposure generally occurs at low levels in the absence of phenotypic changes. Hence, the biological network models, paired with the ability to quantify their activation, will allow derivation of mechanistic fingerprints/signatures for specific materials and their physiochemical properties (particle size, shape, density, surface property, chemical composition), predict tissue toxicity and pathology, find underlying trends in response to exposures, and translate between species (Figure 4B). For example, comparisons at the level of network entities has demonstrated better translatability between experimental systems than differentially expressed genes in the context of cigarette smoke and xenobiotic metabolism [29,30]. Accordingly, network-level comparison of molecular profiles between species could be used to translate preclinical findings to humans. The approach could also provide a robust means to translate between *in vitro* and *in vivo* experiments based on network impact of ENMs of various physical properties and enable bridging to reduce animal use in nanotoxicology.

The limitation of the network approach is that the network models built from literature evidences can only be as complete as our understanding of biology, which is encapsulated in published scientific articles; hence, gaps may exist. Nevertheless, the scoring of omics data onto network models that are built using mechanistic information backed by scientific evidence provides a robust platform for the interpretation of toxicogenomics

contains the molecular function (p, protein abundance; tscript, transcriptional activity; deg, degradation; kin, kinase activity; cat, catalytic activity; bp, biological process) and the namespace definition (MGI, mouse genome informatics; GOBP, gene ontology biological process). The entire cell cycle network model can be downloaded from causalbionet.com, and the BEL terminology can be found in https://bel.bio/. NPA, network perturbation amplitude; BEL, biological expression language; GEO, Gene Expression Omnibus.

Fig. 4



Systems toxicology meta-analysis. **(a)** While conducting meta-analysis, careful data selection is followed by data processing, analysis and interpretation to derive robust conclusions based on multiple experiments. **(b)** The insights gained from meta-analysis in systems toxicology.

data, as demonstrated with the nanoparticle data reanalysis. The approach not only provides a quantitative measure of network perturbation but also allows the inference of the most important molecular players with directionality of the impact.

There are still very few true systems toxicology meta-analyses available, but these are certainly starting to emerge, fueling more accurate toxicity predictions. These will also play an important part in the development of quantitative adverse outcome pathways as well as the reduction of animal use in toxicology.

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Conflict of interest

All authors are employees of Philip Morris International.

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** of outstanding interest

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